Detection of specific antibodies against West Nile and Usutu viruses in healthy blood donors in northern Italy, 2010–2011

A. Pierro[1], P. Gaibani[1], C. Spadafora[1], D. Ruggeri[2], V. Randi[3], S. Parenti[3], A. C. Finarelli[4], G. Rossini[1], M. P. Landini[1] and V. Sambri[5]

1) *Unit of Clinical Microbiology, Regional Reference Centre for Microbiological Emergencies (CRREM), St Orsola-Malpighi University Hospital,* 2) *U.O.C. Idoneità Biologica Donatori Sangue ed Emocomponenti,* 3) *Emilia-Romagna Regional Blood Center,* 4) *Public Health Authority Emilia-Romagna, Bologna and* 5) *Unit of Microbiology, 'AVR – Greater Romagna' Central Laboratory, Pievesestina, Cesena, Italy*

Abstract

Neutralizing antibodies against West Nile (WNV) and Usutu (USUV) viruses were measured in 6000 samples collected, between 1 September 2010 and 30 June 2011, from blood donors living in different districts of Emilia-Romagna, northeastern Italy. On the basis of the microneutralization assay (MNTA), 47 (0.78%) subjects were positive for WNV and 14 (0.23%) for USUV. These results were compared with those obtained 2 years ago and suggest an increased circulation of USUV among humans in Emilia-Romagna.

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Corresponding author: A. Pierro, Unit of Clinical Microbiology, Regional Reference Centre for Microbiological Emergencies (CRREM), St Orsola-Malpighi University Hospital, via Massarenti 9, 40138 Bologna, Italy
E-mail: anna.pierro@aosp.bo.it

Introduction

Usutu (USUV) and West Nile (WNV) viruses belong to the family *Flaviviridae* and are transmitted by mosquitoes, mainly of the genus *Culex* [1].

While the risk represented by WNV infection for human health is well recognized, the medical importance of USUV is not yet fully understood. WNV has circulated in Europe since the 1960s [2–4] and in Italy the first human case of WNV neuroinvasive infection was reported in 2008 [5].

Up to 2009, when the first two neurological cases of human USUV infection were reported in Italy [6,7], this virus was mainly associated with disease in birds [8]. Specific antibody responses against USUV were recently identified among healthy blood donors (BDs) in Italy [9] and in Germany [10]. These findings suggest that USUV is likely to be able to generate a systemic antibody response following asymptomatic infection in humans.

After the first reported cases of human WNV-related disease, a serosurvey study for the identification of anti-WNV antibodies among BDs was planned in northeast Italy from 1 October 2008 to 30 September 2009, demonstrating a seroprevalence of 0.69% [11]. No sample showed neutralizing specific titre antibodies for USUV in this previous study. These results are in agreement with the data regarding the circulation of WNV and USUV in mosquitoes and in birds in the same geographical area during the matching period [12,13].

In the present study the seroprevalence for WNV and USUV among BDs in Emilia-Romagna was investigated 2 years after the first study. The evaluation was performed from 1 September 2010 to 30 June 2011 and involved the districts in the Central-Eastern part of the region (Modena, Bologna, Ferrara, Ravenna, Forlì-Cesena and Rimini). The aim was to investigate the possible change in the seroprevalence ratio against WNV and USUV from 2008–2009 to 2010–2011, comparing two populations of BDs with similar demographic characteristics [11]. This hypothesis was based on the surveillance data provided by the integrated regional system [12–14].

The study cohort included 6000 healthy adult volunteer BDs. Serum samples were collected from all consecutive blood donations from each BD over the study period: once a subject was found positive for WNV antibodies, he/she was not examined further. Written informed consent was obtained from each BD before sampling. All samples were analyzed as previously described [11,15].

One hundred and twenty-five BDs (mean age 45.6 years, range 20–63, male:female ratio 4.4) out of the total evaluated were positive for IgG against WNV by the screening methods, whereas no sample resulted in positive IgM. The IgG-positive samples had a titre ranging from 1/100 to 1/6400, although the most frequent values ranged from 1/100 to 1/400. The IgG avidity was high (>40%) in 117 samples and low (<40%) in the remaining eight specimens (Table 1).

TABLE 1. Detail about the anti-WNV-positive blood donors with low avidity IgG

Sample number	District of residence	IgG-WNV IFA antibody titres	IgM-WNV IFA antibody titres	MNTA titre	IgG-WNV avidity (%)
1	Modena	1/200	Neg	1/10[a]	39
2	Modena	1/100	Neg	$\geq$ 1:640[b]	38
3	Bologna	1/800	Neg	Indeterminate[c]	40
4	Bologna	1/400	Neg	Indeterminate[c]	27
5	Bologna	1/200	Neg	1:320[b]	15
6	Ferrara	1/200	Neg	1:80[b]	32
7	Modena	1/400	Neg	Indeterminate[c]	38
8	Modena	1/100	Neg	Indeterminate[c]	17

MNTA, microneutralization assay; WNV, West Nile virus; IFA, immunofluorescence assay.
[a]Samples with the same MNTA titre against WNV and USUV.
[b]Samples with specific MNTA titre against USUV.
[c]Samples that do not show a specific MNTA titre for either WNV or USUV.

The presence of WNV-specific antibodies was confirmed in 47 samples by MNTA (Fig. 1); 15 of these specimens had a WNV only neutralizing the immune response.

Neutralizing antibodies against USUV were identified in 14 samples (Fig. 1; Table 2), one of which showed neutralizing activity only for USUV.

In the remaining 64 samples that tested positive by the screening assay, it was impossible to establish a specific neutralizing titre: 11 samples had the same neutralizing titre both for WNV and USUV; 53 samples were defined as indeterminate (i.e. the difference between the virus-specific neutralizing immune responses was lower than four times).

Based on the screening results, the WNV seroprevalence was 2.08%; tested sera included in this percentage contained antibodies capable of neutralizing both WNV and USUV.

Considering the MNTA findings, the WNV seroprevalence was 0.78%, being 0.23% for USUV.

In order to compare the results of the present (2010–2011) and the previous (2008–2009, involving only BDs from the area of Ferrara [11]) studies, we paralleled the results obtained only among the BDs living in the district of Ferrara (42 MNTA positive out of the total of 125 in the present study). Twenty-two showed a specific neutralizing titre for WNV (Fig. 1), two showed a specific neutralizing titre for USUV (Fig. 1), and for 18 the neutralizing activity remained not virus specific.

The previous study carried out in 2008–2009 reported a seroprevalence of 0.69% for WNV and no samples showed specific neutralizing antibodies for USUV [11]. The data presented here showed that in the more recent period (2010–2011) the population of healthy BDs developed neutralizing antibodies specific for USUV [10]. On the basis of the MNTA findings obtained in this evaluation, the seroprevalence was 0.78% and 0.23% for WNV and USUV, respectively.

While the seroprevalence obtained for WNV is very similar to the one obtained in the 2008–2009 study [11], the USUV-specific seroprevalence is a new finding.

The results of the present study showed an elevated percentage of samples with quite low titre against WNV. This is probably due to WNV infections that were not recently acquired but probably acquired during the first appearance of WNV in the area in the 2008–2009 season. This hypothesis is also corroborated by the results of the avidity testing, which showed an elevated avidity for 117/125 samples. The MNTA results for the eight samples with low avidity demonstrated a USUV-specific neutralizing activity in three of these specimens (Table 1).

FIG. 1. Map of the Emilia-Romagna region showing the number of blood donors detected positive by MNTA for West Nile (triangle) and Usutu (circle) virus-specific antibody by province. Please note that in this study only six provinces (Modena, Bologna, Ferrara, Ravenna, Forli-Cesena and Rimini) were studied.

TABLE 2. Summary of the characteristics of the 14 blood donors with a USUV-specific antibody response

Sample number	District of residence	IFA IgG-WNV antibody titres	IFA IgM-WNV antibody titres	USUVMNT A titre	WNV IgG avidity (%)
1	Modena	1/100	Neg	≥1:640	38
2	Bologna	1/100	Neg	≥1:640	46
3	Modena	1/200	Neg	≥1:640	42
4	Bologna	1/100	Neg	1/320	48
5	Ravenna	1/100	Neg	1/320	77
6	Bologna	1/100	Neg	1/80	86
7	Bologna	1/100	Neg	≥1:640	49
8	Ferrara	1/200	Neg	1/80	32
9	Modena	1/200	Neg	1/80	79
10	Modena	1/100	Neg	1/80	51
11	Bologna	1/100	Neg	1/80	68
12	Ferrara	1/200	Neg	1/320	86
13	Bologna	1/200	Neg	1/160	99
14	Bologna	1/200	Neg	1/320	15

MNTA, microneutralization assay; IFA, immunofluorescence assay; WNV, West Nile virus; USUV, Usutu virus.

The high percentage of samples with non-virus-specific neutralizing antibodies found in this study may be due to the fact that individuals previously infected with WNV could have subsequently acquired an asymptomatic USUV infection.

In our view, the interesting fact highlighted by this study is the determination, for the first time, of a percentage of seroprevalence for USUV in a large number of healthy subjects. The possibility that the recent spreading of USUV in the Italian population is due to evolutionary divergence of a human-specific virus is under evaluation. Additionally, the seroprevalence data obtained in this study can be used to generate a valuable index of the risk of viral infection for the population living in areas where USUV is spreading. Moreover, the results of this study showed a co-circulation of WNV and USUV in the study area and corroborated the hypothesis regarding USUV infection among humans. These findings, and the recent report of a USUV-specific antibody response among BDs in Germany [10], emphasize the need for monitoring the circulation of both viruses in selected geographical areas.

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Transparency Declaration

The authors declare no conflict of interest.

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